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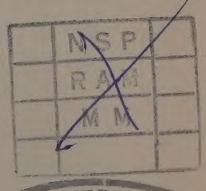

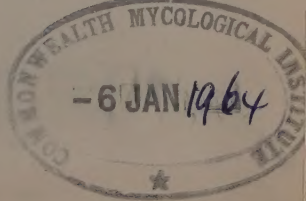

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Change of the activities of oxidative enzymes in potato tubers by the invasion of *Phytophthora infestans*

Masaki Yamamoto, Hiroshi Yasumori, Kadzunori Tatsuyama and Tsutomu Okada

Introduction

A great deal of studies have been made on the nature of late blight resistance of potatoes. The writers' efforts have been given to elucidate the mechanism of resistance of the suscept to the pathogenic fungus, *Phytophthora infestans* from different points of view for these several years[14~24]. Recently, it has become clear that metabolic activity in plant tissue is raised by the infection of pathogens, especially in the tissues resisting against them[1,5,9,10,13].

In the present paper the results of investigations was described on the change of oxidative enzymes and polyphenol content connected with respiration in potato tubers accompanying the invasion of *Phytophthora infestans*.

Materials and methods

Inoculation of pathogen *Phytophthora infestans* (H_1) was cultured on potato decoction agar at 20° C for 30 days and conidial suspension was made with sterilized distilled water. After liberating swarmspores at 12° C this was used as an inoculum. Potato tubers were cut into two pieces, and to one of them the inoculum was smeared with a brush and the other one was kept as a control. These tubers were kept at 20° C until they were used for measuring respiration and oxidase activity.

Measurement of respiration Tubers of Irish Cobbler, susceptible, and Kennebec, resistant were used for the measurement of respiration and the measurements were done every day. Cut slices with the size of 2×1.0 cm ×1.5 mm were prepared from the inoculated and non-inoculated. Five layers of slices were taken until they were at the depth of 10 mm from the surface.

From a tuber, four slices were taken in the same layer, and after measuring fresh weight, the O_2 uptake by respiration was measured by the use of Warburg's manometer.

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In the main chamber of the receptacle, the samples were put with phosphate buffer solution and in the center well 10 per cent KOH solution was placed. After 30 minutes at 30° C, the respiration was measured and the amount of O_2 uptake was represented as O_2 μl/the fresh weight gr/1 hour.

Inhibition of respiration The tuber slices used for the inhibition experiment were the second layer at the depth of 4 mm from the surface. Sodium azide, 8-oxyquinoline, salicylaldoxime, and thiourea were used as inhibitory chemicals at the concentration of 10^{-4} Mol. O_2 uptake was measured by putting these chemicals into manometric vessel. In this case each slice was divided into 4 parts in the thickness of 0.5 mm in order to facillitate the permeation of chemicals to the tubers. The measurement was undertaken on the second and sixth day. The same procedure was also followed in non-inoculated control.

Measurement of catechol oxidase Tuber slices in the fresh weight of 1gr were macerated in a mortar with 5cc of phosphate buffer. After centrifuging, 2cc of supernatant was put in the main chamber of receptacle of the manometer ; 10 per cent KOH solution was put into the secondary chamber, and 1/10 molar catechol was placed into the side chamber of the receptacle. Immediately after such procedure, solutions were mixed with the content of the main chamber and O_2 uptake was measured for 15 minutes at 30 minutes intervals.

Measurement of polyphenol content The similar slices were used for the determination of polyphenol quantity. Five sliced layers were macerated adding 2 per cent metaphosphoric acid in order to inhibit the auto-oxidation of polyphenol within a mortar. Polyphenol was extracted with 50 per cent hot methanol. After filtration, methanol was evaporated and the solution was acidified with sulfuric acid and then filled up to 7cc. One cc of it was taken and put into thiourea and NaN_3 solution. Ten per cent KOH was placed to fill up 4cc of volume. Colorimetric analysis was done using electrophotometer. As a standard, catechol was used in the same procedure as stated above. From the standard curve, the quantity of polyphenol (mg) was calculated.

Experimental results

1) Change of respiration of potato tubers by the infection of *Phytophthora infestans* Change of O_2 uptake in the potato tubers due to the invasion of *Phytophthora infestans* varied with the progress of time and the thickness of infection court. Figures in the table were given in O_2 uptake of infected tubers comparing with that of non-infected tuber slices.

From these results, we can find the raising of respiration from the first day in the resistant variety comparing with that of non-inoculated control and the raising showed its maximum about two-fold strength of the control on the third day. However, remarkable increase was not found lower than the third layer. In the susceptible variety, the respiration raised on the 4th day and the increase was also found in the deeper places, and this tendency was remarkable on the 6th day, and there was no tremendous difference of the maximum of raising ratio between the two varieties.

Table 1 Change of respiration in potato tubers infected with *Phytophthora infestans*

Variety	Days after inoculation	Respiration				
		2*	4	6	8	10
Irish Cobbler	1	1.20	1.09	1.08	1.10	0.85
	2	1.01	1.07	1.24	1.25	0.96
	3	1.21	1.21	0.92	0.95	0.89
	4	1.13	2.22	1.40	1.45	1.20
	5	1.19	1.92	1.57	1.50	1.07
	6	1.03	2.19	1.97	1.80	1.63
Kennebec	1	1.17	1.42	——	1.31	0.96
	2	1.14	1.54	0.86	1.13	1.16
	3	1.16	2.09	1.62	1.18	——
	4	1.18	1.83	1.27	1.24	1.22
	5	1.12	1.40	1.64	1.40	1.37
	6	1.19	1.60	1.40	1.31	1.23

N. B. * Distance from the inoculated surface (mm)
Figures are given as a relative value, when control (O_2 uptake in non-inoculated tubers) is shown as 1.00

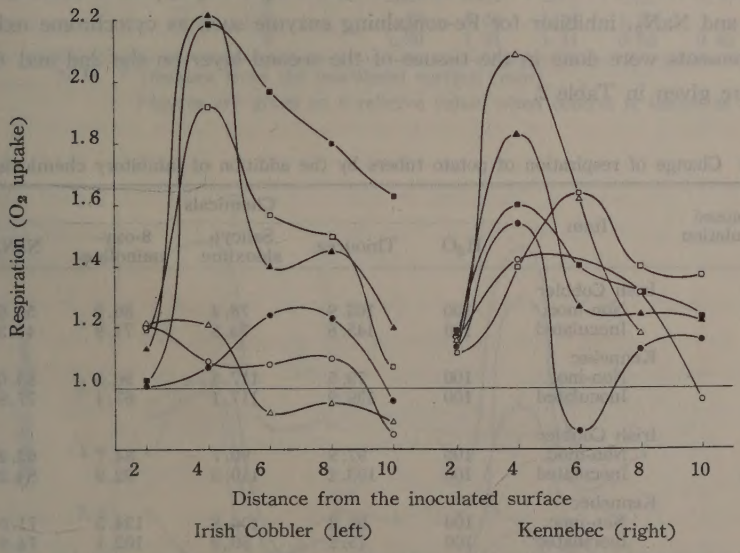

Fig. 1 Change of respiration in potato tubers infected with *Phytophthora infestans*

N. B. —○— First day after inoculation
—●— Second day after inoculation
—△— Third day after inoculation
—▲— 4th day after inoculation
—□— 5th day after inoculation
—■— 6th day after inoculation

In the susceptible variety, the advancement of mycelia arrived at the boundary of 1st and 2nd layers on the 3rd day, on the 4th day in the middle of second layer, in the third layer on the 5th day, and in the 4th layer on the 6th day. Partial browning occurred in the third layer on the 6th day. Yellowing (poisoning), however, occurred in general tissue

in one more deeper layer than the browned part. Accordingly, the yellowing was found in the 4th-5th layer on the 6th day. Meanwhile, in the resistant variety, the invasion of the fungus was restricted, and no advancement was found in the deeper parts, but the surface browned severely and the yellowing reached to the middle of the second layer on the 6th day.

In regard to fungus invasion, the raising of respiration in the susceptible variety was found in the part in which the mycelium already invaded. However, in the resistant variety, the maximum respiration was mentioned in the poisoning part in which there was no invasion of the fungus. In other words, the rapid response is likely to occur in the tissue of resistant variety accompanying the velocity of fungus invasion.

2) Inhibition of respiration The respiration of the infected tuber tissues raised about two-fold at its maximum comparing with that of non-inoculated tubers. However, it is quite uncertain what kinds of respiratory enzymes are concerned to the respiratory raising. In order to make clear these points the O_2 uptake was measured under the influence of various inhibitory chemicals to respiration. Chemicals used were thiourea, 8-oxyquinoline, and salicylaldoxime, inhibitor for Cu-containing enzymes such as polyphenol oxidase, ascorbic acid oxidase, and NaN_3, inhibitor for Fe-containing enzyme such as cytochrome oxidase.

Measurements were done in the tissues of the second layer on the 2nd and 6th day. The results are given in Table 2.

Table 2 Change of respiration of potato tubers by the addition of inhibitory chemicals

Days elapsed after inoculation	Item	Chemicals				
		H_2O	Thiourea	Salicyl-aldoxime	8-oxy-quinoline	NaN_3
2	Irish Cobbler					
	Non-inoc.	100	105.9	78.4	86.0	55.6
	Inoculated	100	145.8	73.8	71.9	41.3
	Kennebec					
	Non-inoc.	100	79.5	117.4	90.5	83.0
	Inoculated	100	139.9	117.1	87.4	77.9
6	Irish Cobbler					
	Non-inoc.	100	97.9	90.7	84.7	63.2
	Inoculated	100	103.2	110.3	92.9	84.2
	Kennebec					
	Non-inoc.	100	98.8	106.8	134.3	71.0
	Inoculated	100	75.2	80.2	102.1	74.9

In all plots, the inhibition of respiration by NaN_3 was especially remarkable. As a rule, on the second day after the inoculation, the respiration of inoculated tubers was less than that of non-inoculated ones. On the sixth day, however, the inhibition degree decreased and the respiration of inoculated tubers showed greater value than in non-inoculated ones. The same tendency might also exist in the respiratory change of tubers by the addition of inhibitory chemicals of Cu-containing enzymes, but a definite conclusion must be postponed till the further studies will be completed.

From these considerations, the activity of metal enzymes including such as Cu and Fe were revealed in the tissues invaded by the fungus and in the susceptible variety, the

activity decreased gradually and the possibility of the other enzyme system besides iron-and copper-containing enzymes might have the opportunity to decrease too.

3) Change of catechol oxidase The change of the activity in respective layers with the progress of time is given in Fig. 2.

Table 3 Change of catechol oxidase of potato tubers due to the invasion of *Phytophthora infestans*

Variety	Days after inoculation	Activity of catechol oxidase				
		2*	4	6	8	10
Irish Cobbler	1	1.20	1.01	——	1.05	0.92
	2	1.39	1.66	1.11	0.92	0.92
	3	1.56	1.62	1.07	1.12	0.99
	4	2.03	2.07	1.47	1.49	——
	5	1.50	1.58	1.01	1.20	1.18
	6	——	——	——	——	——
Kennebec	1	1.00	1.14	0.85	0.91	1.04
	2	1.25	1.17	——	1.00	0.89
	3	1.02	1.90	1.40	1.41	——
	4	——	——	——	——	——
	5	1.29	2.06	1.28	1.39	——
	6	1.20	1.55	1.11	0.86	0.86

N. B. * Distance from the inoculated surface (mm)
Figures are given as a relative value, when control is shown as 1.00

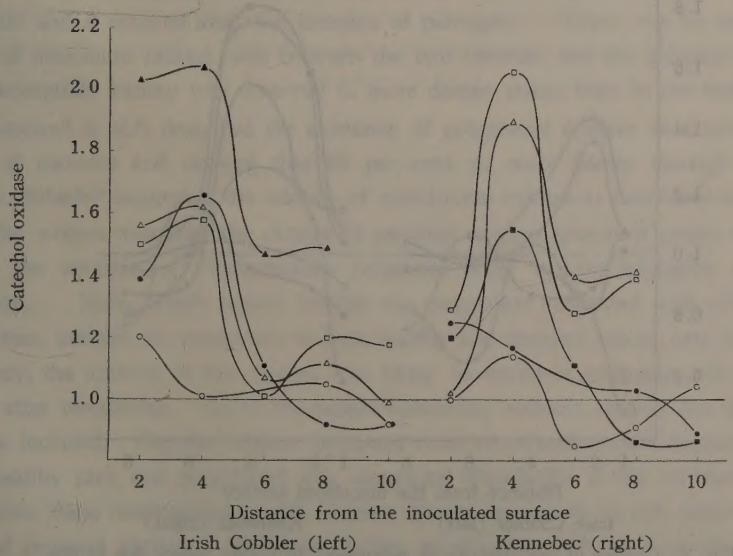

Fig. 2 Change of catechol oxidase in potato tubers infected with *Phytophthora infestans*

N. B. —○— First day after inoculation
—●— Second day after inoculation
—△— Third day after inoculation
—▲— 4th day after inoculation
—□— 5th day after inoculation
—■— 6th day after inoculation

The activity of the enzyme was highest in the 2nd layer in both susceptible and resistant varieties, and in the susceptible variety raising of the activity was not so remarkable in the deep place as was seen in the case of respiration, and the activity in the first layer seem to be higher in comparison with that of the resistant variety. Accordingly, the activity

Table 4 Change of polyphenol content due to the invasion of *Phytophthora infestans*

Variety	Days elapsed after inoculation	Polyphenol Index compared with non-inoculated plot (1.00)				
		* 1	2	4	6	8
Irish Cobbler	1	1.06	0.82	1.03	0.63	0.83
	2	1.01	0.89	0.87	1.06	0.76
	3	1.17	1.44	1.18	0.97	1.04
	4	1.07	1.36	1.06	0.96	0.95
	5	0.49	0.82	0.97	——	1.00
	6	——	——	——	——	——
Kennebec	1	0.90	0.97	1.03	1.03	0.95
	2	0.80	0.89	0.96	1.17	0.77
	3	1.08	1.59	1.75	1.11	1.00
	4	0.59	1.12	1.28	0.90	0.88
	5	1.14	1.17	1.55	0.96	0.80
	6	1.04	1.58	1.80	1.00	0.96

* Distance from the inoculated surface (mm)

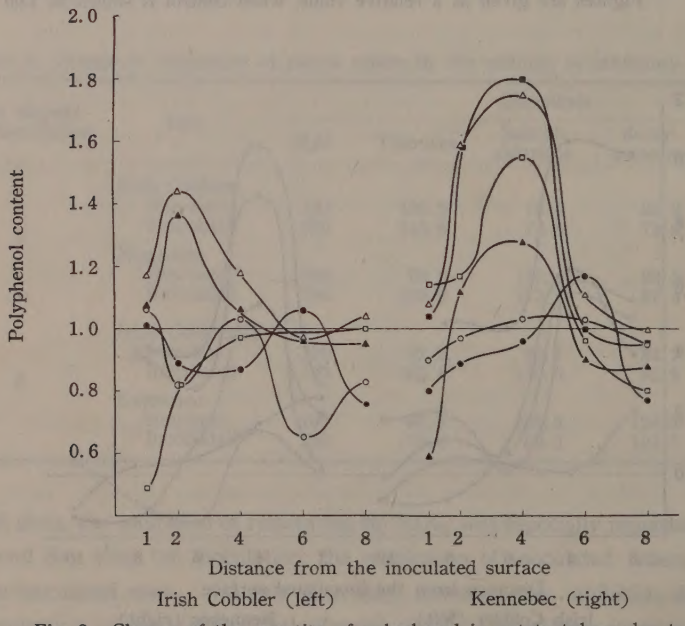

Fig. 3 Change of the quantity of polyphenol in potato tubers due to the invasion of *Phytophthora infestans*

N. B. —○— First day after inoculation
—●— Second day after inoculation
—△— Third day after inoculation
—▲— 4th day after inoculation
—□— 5th day after inoculation
—■— 6th day after inoculation

of this enzyme might be enhanced in the tissues in which the mycelial density was highest or in the tissues in which the pathogen had already invaded than in the poisoning parts. However, in the resistant variety, Kennebec, raising of the activity was observed in the poisoning part in which the fungus was not yet invaded.

4) Change of polyphenol content The change of the quantity of polyphenol in tubers of both resistant and susceptible varieties was shown in Fig. 3 and Table 4.

In the resistant variety, at the depth of 4 mm of tuber, the quantity of polyphenol increased with the progress of time, but in the susceptible variety, the quantity increased at the depth of 1 mm and decreased with the progress of time comparing with the non-inoculated plot.

Discussion

There are many reports regarding the increase of respiration in the suscept tissues invaded by the pathogen[1,3,4,6], and especially much discussion had been given about the mechanism of increasing respiration by Sempio[6], Allen[1] and Walker[4]. Tomiyama et al.[11,12] stated on the respiration of potatoes infected with *Phytophthora infestans* and they described the increase of water soluble proteins, starch and polyphenol content in tubers.

From the results of the writers' experiments, the resistant variety showed the rapid raising of respiration in the tissues in which there was not yet the invasion of pathogen, however, in the susceptible variety, it required a rather long time to see the raising of respiration and it occured after the invasion of pathogen. There was no remarkable difference of maximum raising ratio between the two varieties and the increase of respiration in the susceptible variety was observed in more deeper places than in the resistant variety.

Bosewell et al.[2] described the existence of polyphenol oxidase as a terminal enzyme system of potatoes and showed that 85 per cent or more passes through this system. However, Schade[5] suggested the relation of cytochrome oxidase as a terminal oxidase system.

The writers measured the change of terminal oxidase system of potato tubers accompanying the invasion of *Phytophthora infestans* using various inhibitory chemicals for respiration. NaN_3 which mainly inhibits the respiration connected with cytochrome oxidase system, inhibits the respiration in both healthy and diseased tissues, and in the susceptible variety, the activity of this system was likely to decrease gradually with the progress of time after inoculation. As to the copper-containing enzyme system, the same tendency might be included. Catechol oxidase increases more remarkably in the inoculated part than in the healthy part, and polyphenol was recognized abundantly in the resistant variety.

From these observations, metal enzyme system seemed to be still active in the tuber tissues of resistant variety. However, further experiments will be required to determine whether Cu- or Fe-containing enzyme might be connected with the resistance and/or moving to other system.

In the writers' experiments, the quantity of polyphenol increased with the progress of time at the depth of 4 mm of tuber in the resistant variety, but in the susceptible variety the quantity increased at the depth of 1 mm. It has already known that the necrosis of

an injured cell has a close connection with oxidation of polyphenol compounds. Rubin and Aksenova[7] stated that infection of stable form of potatoes is followed by an increased activity of polyphenol oxidase, and the distortion of the normal course of respiration in stable forms is the direct course of accumulation of quinones. Johnson and Schaal[6] reported that the content of polyphenol compounds, especially chlorogenic acid had to do with the resistance of potato plants to scab. Tomiyama et al.[11] indicated that infection of thin slices with incompatible races gives the least polyphenol content and that an increase of polyphenol content was observed in case of resistant reaction.

Summary

1. In this paper the change of an activity of oxidative enzymes connected with respiration in potato tubers accompanying the invasion of *Phytophthora infestans* was dealt with.

2. The respiration of tissues invaded by the pathogen increased and the increase was rapid in the resistant variety, and in the susceptible variety, the increase of respiration continued to deeper places gradually.

3. Respiration in both healthy and diseased tissues was inhibited by the addition of various chemicals and the inhibition was remarkable with NaN_3. In the susceptible variety, the ratio of inhibition decreased on the 6th day after inoculation.

4. The activity of catechol oxidase increased remarkably at the inoculated places.

5. Polyphenol was recognized in the poisoning part of the resistant variety.

6. The physiological change stated above showed highest value at the depth of 2~4 mm from the inoculated place.

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疫病菌侵入に伴う馬鈴薯塊茎中の酸化酵素活性の変化

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摘 要

1. 疫病菌侵入に伴う馬鈴薯塊茎の呼吸系酸化酵素の変動をしらべた.
2. 菌侵入により組織の呼吸は増大し，抵抗性品種においてその増大は速やかであつた．また罹病性品種では呼吸の上昇は深部迄及んだ.
3. 呼吸阻害剤により，健全・病組織ともその呼吸は抑制され，特に NaN_3 によるものが著しかつた．しかし罹病性品種では接種後6日目で抑制率はやや減じた.
4. カテコールオキシダーゼは接種部でやや減じた.
5. ポルフェノールは抵抗性品種で中毒部に多く認められた.
6. 以上の生理的変動は接種面より 2～4mm の深さで最も顕著であつた.

Shokubutsu Byogai Kenkyu, Kyoto 6(4): 126~131, 1959

Biochemical studies on *Cochliobolus miyabeanus*

II. Enzymes concerning amino acid utilization

(Part 2) On the transaminase and the amino acid decarboxylase

Hachiro Oku*

Introduction

Since *l*-amino acid oxidase has not been found in *Cochliobolus miyabeanus*[10], an expected relationship between the amino acid utilization and amino acid oxidase should be denied. The present report dealt with the investigation of the activities of transaminases and amino acid decarboxylases of this fungus.

Methods

Preparation of enzymes

1) Transaminase Mycelium of *Cochliobolus miyabeanus* was inoculated on the modified Czapek medium[10] in shaking-flasks and raised for 64 hours at 26°C. Mycelium was harvested by filtration, washed with distilled water, weighed and macerated in a Waring blendor cup with 3 volume of M/150 phosphate buffer (pH 6.9) at 0°C. The resulting cream was centrifuged for 5 minutes at 4000 r p m, and the supernatant was dialysed at 1~5°C for overnight against the same buffer as was used in the extraction. The resulted solution was used as enzyme solution of transaminase.

2) Amino acid decarboxylase Acetone dried mycelium was prepared as described in elsewhere[10].

Measurement of enzyme activities

1) Transaminase activity Measurement of transaminase activity was carried out by Fincham's method of paper chromatography[5] with several modifications.

The formation of glutamic acid or alanine by the incubation of enzyme solution with α-ketoacids and various amino acids was determined by paper chromatography. The running solvent, *n*-Butanol: acetic acid: water (4:1:1) was used.

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The glutamate and alanine obtained were roughly estimated by visual comparison of the experimental spots with graded series of concentration of glutamate and alanine standards chromatographed under the identical conditions. Glutamate and alanine concentrations were recorded as either "intermediate" between two standards or as "equal" to one of the standards when they could not be seen to differ from it in intensity.

2) Activity of amino acid decarboxylase CO_2 liberation was measured using Warburg manometric technique at 30° C. Each manometer vessel contained 2.5 m*l* of M/15 phosphate buffer and 50mg of acetone dried mycelium in the main cup and 0.5m*l* of M/15 *l*-amino acids in the side arm. The gas phase was air. In all experiments the small autorespiration was corrected by placing enzyme suspension in the thermobarometer vessel.

Results

Transaminase

1) Transaminase activities in *C. miyabeanus* As was shown in Table 1, alanine, aspartate, valine, and glutamate were transaminated in 3 hour's incubation. Glutamate-pyruvate transaminase was most active in this fungus. But no transamination was observed with any other amino acids.

Table 1 Transaminases in *C. miyabeanus*

Substrate	-NH_2-acceptor	
	Pyruvate	α-Ketoglutarate
	Alanine formed (mM)	Glutamate formed (mM)
None	0	0
Glycine	0	0
l-Alanine	——	5
l-Leucine	0	0
l-Arginine-HCl	±	0
l-Threonine	0	0
l-Proline	0	0
l-Methionine	±	0
l-Histidine-HCl	0	0
l-Phenylalanine	0	0
l-Glutamic acid	18	——
l-Aspartic acid	0	3
l-Lysine-HCl	0	0
dl-Serine	0	0
dl-Valine	3～5	0

Test-tubes each containing: 0.45 m*l* of *l*-amino acid, 0.15 m*l* of M/5 sodium-α-ketoglutarate or sodium pyruvate dissolved in M/15 phosphate buffer at pH 6.9, and 0.4 m*l* of mycelial extract
Incubation: at 35° C for 3 hours

2) Optimum pH of transaminase The effect of pH on the transaminase activity was determined with glutamate as a amino-donator and pyruvate as an acceptor in M/15 phosphate buffer solution.

The optimum pH was 7.0 to 8.0 (Fig. 1).

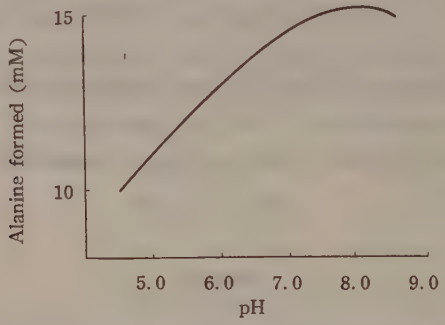

Fig. 1 Effect of pH on the glutamate-pyruvate transaminase of *C. miyabeanus*

3) Inhibitors of transaminase The inhibition experiments were carried out with glutamate-pyruvate transaminase. As shown in Table 2, 10^{-3}M $AgNO_3$, 10^{-3}M $HgCl_2$, 2×10^{-2}M iodoacetic acid, 2×10^{-2}M malonate, 10^{-2}M hydroxylamine, and 10^{-2}M semicarbazide-HCl were found to inhibit its activity completely. No inhibition was observed with 2,4-dinitrophenol.

Table 2 Effect of inhibitors on the glutamate-pyruvate transaminase of *C. miyabeanus*

Inhibitor	Concentration M	Inhibition %
$AgNO_3$	10^{-3}	100
	10^{-4}	84
$HgCl_2$	10^{-3}	100
	10^{-4}	84
$CuSO_4\cdot5H_2O$	10^{-3}	60
Hydroquinone	10^{-3}	75
2-Methyl-1, 4-naphthoquinone	10^{-3}	0
$NaAsO_2$	2×10^{-2}	75
CH_2JCOOH	2×10^{-2}	100
	10^{-3}	55
Malonic acid	2×10^{-2}	100
	10^{-3}	35
NH_2OH-HCl	10^{-2}	100
	10^{-3}	45
Semicarbazide-HCl	10^{-2}	100
	10^{-3}	0
KCN	10^{-3}	55
2,4-Dinitrophenol	10^{-3}	0
Actidione	100γ/ml	0
Ophiobolin	10γ/ml	0

Amino acid decarboxylase

Only *l*-glutamic acid decarboxylase activity was found in the enzyme preparation of *C. miyabeanus* (Table 3).

Table 3 Amino acid decarboxylases in the acetone dried mycelium of *C. miyabeanus*

Amino acid	CO_2 release (m*l*/30min)
l-Alanine	0
l-Aspartic acid (Na)	0
l-Arginine-HCl	0
l-Glutamic acid (Na)	96.5
l-Histidine-HCl	0
l-Leucine	0
l-Lysine-HCl	0
l-Phenylalanine	0
l-Proline	0
l-Tyrosine	0
dl-Tryptophan	0
l-Methionine	0

The optimum pH of *l*-glutamic acid decarboxylase was found to be 5.5 (Fig. 2).

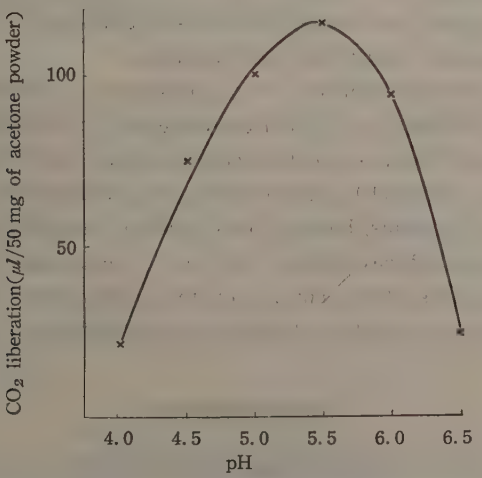

Fig. 2 Effect of pH changes on the glutamic acid decarboxylase of *C. miyabeanus*

l-Glutamic acid decarboxylase of this fungus was completely inhibited by 10^{-4}M hydroxylamine, 10^{-3}M KCN, 10^{-3}M $HgCl_2$, 10^{-3}M $AgNO_3$, and 10^{-2}M monoiodoacetic acid. It is interesting that ophiobolin inhibited this enzyme activity at a concentration of 100γ/m*l*, since ophiobolin is a toxic substance produced by *C. miyabeanus*.

Table 4 Effect of inhibitors on the glutamic acid decarboxylase of *C. miyabeanus*

Inhibitor	Concentration (M)	Inhibition (%)
CH_2JCOOH	10^{-2}	100
	10^{-4}	0
NH_2OH-HCl	10^{-2}	100
	10^{-4}	100
	10^{-5}	26
iso-Nicotinic acid hydrazide	10^{-3}	48
KCN	10^{-3}	100
$HgCl_2$	10^{-3}	100
$AgNO_3$	10^{-3}	100
$NaHSO_3$	10^{-2}	100
Na-acetate	10^{-2}	39
Actidione	100γ/m*l*	0
Ophiobolin	100γ/m*l*	55
Ethanol	10%	30

Discussion

Among the three enzymes tested, amino acid oxidase, transaminase, and amino acid decarboxylase, only transaminase seemed to play a part on the amino acid utilization of *C. miyabeanus*. However, since the enzyme preparation of this fungus transaminated only alanine, glutamate, aspartate, and valine, the means of utilization of other amino acids such as *l*-proline, *l*-arginine, and *l*-leucine were still unknown.

The natures of the transaminase of *C. miyabeanus* seemed to differ from other origin's[1,2], which was inhibited by 10^{-3}M iodoacetate and 2×10^{-2}M arsenate.

Bacteria have been shown to contain decarboxylases for various amino acids[3,4,6,7], and decarboxylases for histidine[12], tyrosine[8], dopa[9], tryptophane[13] have been found in animal tissues. In higher plants, however, only glutamic decarboxylase has been found[11].

The only amino acid decarboxylase found in *C. miyabeanus* was also glutamic decarboxylase, and the natures of the enzyme of this fungus really resembled to the same enzyme in higher plants, in regard to pH optimum and inhibition experiments.

Acknowledgment

The author wishes to express his appreciation to Prof. S. Akai, Kyoto University, Mr. M. Matsui, chief director of this laboratory, and Mr. Y. Miura of this laboratory, for their advices and encouragements.

Summary

1) Glutamate-pyruvate, aspartate-ketoglutarate, valine-pyruvate, and alanine-ketoglutarate transaminases were found in *C. miyabeanus*.

2) Transaminase of this fungus was strongly inhibited by iodoacetate, hydroxylamine and heavy metals.

3) The optimum pH of transaminase of this fungus was found to be 7.0 to 8.0.

4) The only one amino acid decarboxylase, glutamic decarboxylase, was found in this fungus, and the natures of this enzyme really resembled to the higher plants origin's.

5) Among the three enzymes tested, transaminases seemed to play a part on the amino acid utilization of *C. miyabeanus*.

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稲紋枯病菌菌糸の伸長に対する 2,4-D の影響について

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Kaoru Kurodani, Kuniomi Yokogi and Masaki Yamamoto:
On the effect of 2,4-dichlorophenoxy acetic acid to the mycelial growth of *Hypochnus sasakii* Shirai

1. 緒　　言

2,4-D 散布は水田の雑草駆除の目的で行われているが，その散布が作物の病害感受性に影響をおよぼすという観察も少なくない．山仲[11]は 2,4-D 散布が稲胡麻葉枯病の発生を減少せしめることを，また深津[2]は稲小粒菌核病その他の発生を減少せしめることを報告した．内藤[7,8]は，ペプトン加用合成培地に種々濃度の 2,4-D 液を混入し，それにいろいろの植物病原菌を培養して菌糸の生長量をしらべ，抑制されるものと，促進されるものとを区別した．また，赤井[1]らは種籾の 2,4-D 処理と水稲成葉の胡麻葉枯病発生との関係を調べ，感受性の低下を示すことを報じている．2,4-D その他の植物ホルモンの，植物病原菌におよぼす影響については，内藤[9]の詳細な研究がある．

筆者らは，2,4-D 散布と稲紋枯病発生の関係について 1954～55年に行つた実験結果につき報告する．本研究に関し御助言をたまわつた嵐農林省四国農業試験場長，高野島根農大教授，また稲紋枯病菌を分譲された赤井京大教授に深謝の意を表する．

2. 稲葉鞘部に 2,4-D を散布した場合の稲紋枯病菌の感受体侵入におよぼす影響

(1) 実験材料および方法　水稲農林44号を1955年 6 月22日，5 万分の 1 ワグネルポットに 1 本植とし，8 月12日に生育状態のほぼ同じもの（草丈80 cm，葉位12，分蘖16）を10茎残し，その他は切り取つた．葉鞘に 0.05% 2,4-D (Na 塩) を霧吹きで一定量散布し，1 昼夜後，最下位葉鞘の葉舌下 2cm の内側に予め扁平培養によつて生じた紋枯病菌核（菌核形成後 7 日のもの）の大きさ，色ともにほぼ等しいものを接種した．その後 2 日置きに一定時刻に観察して病斑の進展程度を調査した．病斑の大きさは直径の最大の所を直角の 2 方向に測り，それを乗じたものであらわした．また病斑数は 1 茎に現われた全部のものを算えた．なお無散布区は水を 2,4-D 液の代りに散布し，各回の実験に10茎を用い，各実験は 4 回反覆した．

第 1 表　稲紋枯病菌の侵入におよぼす 2, 4-D の影響

実験回数	処理区別	接種後の日数 2 病斑の大きさ L×S	2 1茎当病斑数	4 病斑の大きさ L×S	4 1茎当病斑数	6 病斑の大きさ L×S	6 1茎当病斑数	8 病斑の大きさ L×S	8 1茎当病斑数
		cm^2		cm^2		cm^2		cm^2	
Ⅰ	標準区	4.86	0.90	12.72	2.60	15.87	3.40	18.28	4.40
	処理区	2.94	1.10	8.78	2.80	16.88	5.30	21.56	6.60
Ⅱ	標準区	2.51	1.60	12.23	3.60	18.28	4.90	25.62	5.70
	処理区	3.50	1.30	13.49	3.00	21.36	4.30	22.45	5.60
Ⅲ	標準区	1.20	0.70	6.95	2.00	10.60	3.10	12.60	4.00
	処理区	3.04	0.80	11.20	2.70	19.09	5.10	24.04	6.20
Ⅳ	標準区	1.47	0.80	6.93	2.40	15.76	4.60	20.64	5.60
	処理区	2.36	1.30	10.33	2.63	18.73	4.80	25.80	6.90
平均	標準区	2.50	1.00	9.70	2.65	15.10	4.00	17.64	4.92
	処理区	2.96	1.10	10.95	2.77	19.00	4.90	23.66	6.32

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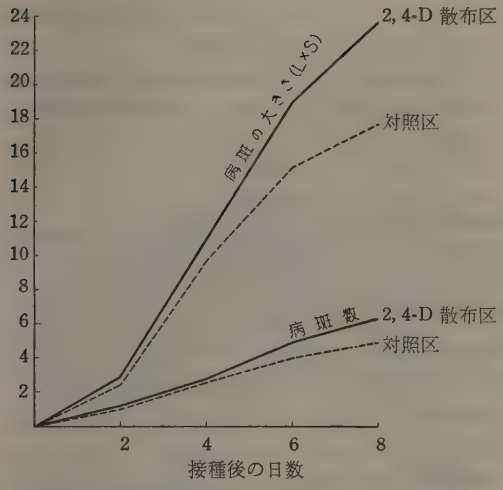


第1図　稲紋枯病菌の侵入におよぼす 2,4-D の影響

(2) 実験結果　第1表および第1図に示す如く 2,4-D 散布区，標準区ともに接種後日数の経過に伴つて病斑の大きさおよび数は増加しているが，2,4-D 散布区は標準区よりも病斑の大きさ数ともに常に大きい．両者の差は0.05%の危険率において有意差を認めた．

3. 病斑内菌糸の病原性におよぼす 2,4-D の影響

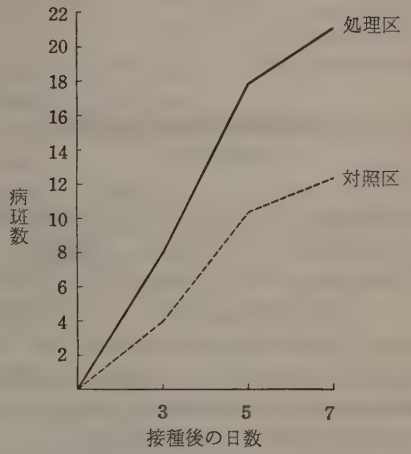
実験2において，2,4-D 散布区では標準区にくらべ稲紋枯病の発生が大になつた結果から，病斑中の菌糸が 2,4-D によつて，どのような影響を受けるかがつぎの問題として浮び上がる．この点を解明しようとしてこの実験を行つた．

(1) 実験材料および方法　水稲農林1号を1955年6月20日，6寸鉢に1本植とし，8月7日生育状態の同程度のものを1鉢5茎残して刈り取つた．発病後4日を経た病斑（0.5 cm×0.5 cm）を切り取つて葉鞘内に接種し，3，5，7日後に，病斑の大きさおよび数を調査した．接種後3日間ビニールの覆をし，28～30°C，関係湿度90%に保つた．1回の実験に10茎を用い，5回反覆実験した．

第2表　病斑中の菌糸の病原性におよぼす 2,4-D の影響

実験回数	処理区別	接種後の日数 3 病斑数	5 病斑の大きさ L×S	5 病斑数	7 病斑の大きさ L×S	7 病斑数
			cm^2		cm^2	
I	対照区	5.00	8.30	16.00	8.80	17.00
	処理区	5.00	9.10	14.00	12.10	19.00
II	対照区	4.00	5.80	10.00	6.00	11.00
	処理区	7.00	8.80	16.00	10.80	17.00
III	対照区	3.00	4.40	8.00	5.40	9.00
	処理区	12.00	18.80	23.00	21.00	27.00
平均	対照区	4.00	6.16	11.30	6.73	12.30
	処理区	8.00	12.20	17.60	14.60	21.00

(2) 実験結果　対照区と処理区との差は，0.05%の危険率で有意差を認めた．第2表，第2図に示す如く，処理区は標準区より明らかに病斑の大きさ，数ともに大きくなつている．すなわち 2,4-D を散布した水稲内の稲紋枯病病斑内菌糸の病原性は，無散布のものより大きいことがわかつた．



第2図　病斑中の菌糸の病原性におよぼす 2,4-D の影響

4. 稲紋枯病病原菌菌糸の伸長におよぼす 2,4-D 濃度の影響

内藤[7,8]は，各種濃度の 2,4-D 液をペプトン加用合成培地に入れ，菌糸の発育程度を調査しているが，著者らは稲体煎汁を用いて 2,4-D を混入した場合の稲紋枯病菌の発育状態をしらべた．

(1) 実験材料および方法　稲紋枯病菌を培地に植付けて6日後菌核を形成させたものを供試菌とした．水稲農林44号を挿抜したものが草丈 48～50cm，葉位12，分蘖11程度になつたとき葉鞘部に濃度を異にした 2,4-D 液を霧吹きで一定量散布した．一昼夜後その部分を蒸溜水でよく洗滌したもの(A)と，そのままのもの(B)おのおのの生体重25gr 宛を切り取り，蒸溜水 200cc とともに40分間煮沸して得た煎汁に2%の寒天を加え，5～6 lb で20分間殺菌した．それをペトリ皿に 10cc 宛注加し，稲紋枯病菌の菌核のほぼ等大のものを植付け，28～30°C に5日間保ち，発育した菌叢の直径を直角の2方向に測定して，その平均値をもつて菌糸の伸長をあらわした．1回の測定には各区6枚のペ

トリ皿を使用した.

(2) 実験結果　第3表に見る如く 2,4-D, 5×10^{-2}, 5×10^{-1}%の濃度では稲紋枯病菌菌糸の伸長は非常に大きかつた. 内藤[7,8]の結果では2×10^{-2}%の濃度で菌糸の伸長量は促進されたが, 著者らの場合5×10^{-1}%の濃度でも抑制効果はあらわれていない. おそらく 2,4-D の濃度が実際にはさらに稀釈されているためであろう.

A, B両区間, すなわち 2,4-D を散布後, 葉鞘面を蒸溜水で洗滌したものと, しないものとでは, 明らかな差が認められなかつた.

第3表　2, 4-D を散布した水稲の煎汁が稲紋枯病菌菌糸の伸長におよぼす影響

	2, 4-D 散布, 葉鞘面を洗滌した場合					葉鞘面を洗滌しない場合				
濃度別 %	対照区	5×10^{-4}	5×10^{-3}	5×10^{-2}	5×10^{-1}	対照区	5×10^{-4}	5×10^{-3}	5×10^{-2}	5×10^{-1}
菌叢直径 平均 mm	70.62	64.00	68.60	82.80	79.13	68.20	64.38	70.30	87.30	83.30

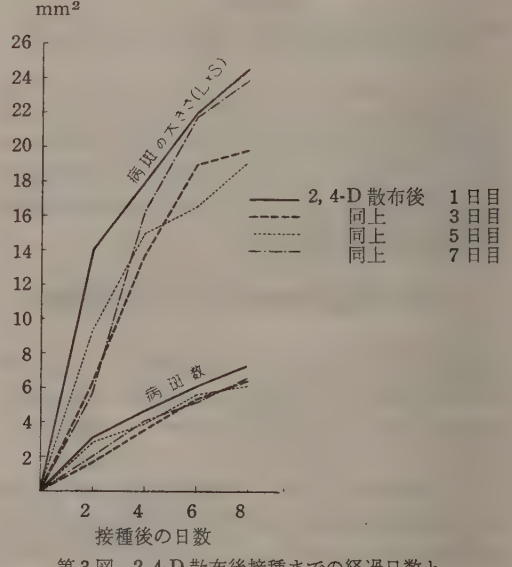
5. 2,4-D 散布後の経過日数と稲紋枯病の発生について

稲葉鞘部に 2,4-D が散布されてからの経過日数と稲紋枯病の発生との関係を明らかにするため, 本実験を行つた.

(1) 実験材料および方法　水稲農林44号を5万分の1ワグネルポットに6月22日に1本植とし, 8月23日分蘖が16本となつたとき生育が同程度のもの10茎だけを残して他は地際から切り取り, 5×10^{-2}%の 2,4-D 液を一定量葉鞘部に霧吹きで散布した. 生育期間中各ポットに硫酸アンモニア, 過燐酸石灰おのおの 1gr 宛を2回追肥した.

2,4-D 散布後, 1, 3, 5, 7日目に, 稲紋枯病菌の3日培養の菌叢を 0.5cm² に切抜いて, 最下位葉鞘の葉舌下 2cm のところに接種して, その後2, 4, 6, 8日目の一定時刻に病斑の大きさ, 数を調査した.

(2) 実験結果　第4表および第3図に見る如く, 2,4-D 散布後1日目に接種したものは3, 5, 7日目に接種したものより大きさ, 数共に大きいように思われたが, 散布後経過日数と稲紋枯病の発生との関連性については, 今後の研究を要する.



第3図　2, 4-D 散布後接種までの経過日数と病斑の大きさと数

第4表　2, 4-D 散布後接種までの経過日数と病斑の大きさ（単位 cm²）および日数との関係

2, 4-D 散布後日数	接種後日数 2		4		6		8	
	病斑大きさ	数	病斑大きさ	数	病斑大きさ	数	病斑大きさ	数
1	14.00	3.00	18.00	4.70	22.00	6.00	24.50	7.20
3	6.30	1.70	13.70	3.60	19.00	5.30	19.90	6.40
5	9.40	2.90	15.00	4.00	16.40	5.50	19.20	6.20
7	5.90	2.00	16.30	4.10	22.00	5.10	24.00	6.60

6. 考　　察

2,4-D が一般植物に対して選択性の殺草機能を有するのと同様，植物病原菌類に対しても，ある種のものに選択性を持つていることが内藤[6]らの研究によつて明らかである．山仲[11]，内藤ら[7,8]は，稲の茎葉に 2,4-D を散布した場合，稲胡麻葉枯病の発生を減ずることを報告し，鳥取農試[9]の圃場試験結果では昭和26年度は発病を助長し，27年度は却つて抑制している．著者らの実験では稲紋枯病菌は 2,4-D によつて菌糸の伸長を促進せられたが，稲葉鞘部に 2,4-D を散布した場合に発生した病斑内の菌糸は，無散布部に発生した病斑内の菌糸よりも病原性が大であつた．

横木ら[12]は，2,4-D 散布後24時間目に稲紋枯病菌菌糸を接種したところ，著者らの結果と同様に，2,4-D 散布区は発病を明らかに助長したが，同一濃度の 2,4-D 液を接種後24時間目に散布した場合には，以後の病勢進展が明らかに抑制せられる傾向を認めた．また圃場試験においても早期散布区はやや発病を助長し，後期散布はやや抑制する傾向がうかがわれた．

稲紋枯病菌菌糸の伸長におよぼす 2,4-D の濃度の影響は内藤[6,7]の結果と同様で，対照区よりも処理区において菌糸伸長量は大であつた．

除草，殺草の目的で 2,4-D 散布がなされるが，稲紋枯病の発生時期と大体一致することが多く，以上の実験結果よりみて散布の時期は，かなり問題となつて来るものと考えられる．

摘　　要

1．2,4-D (Na 塩 5×10^{-2}%) の葉鞘散布によつて，稲紋枯病は標準区より明らかに病斑の大きさ，数ともに増加した．

2．2,4-D 散布区の病斑内菌糸のほうが無散布区のそれに比して病原性が強かつた．

3．2,4-D を散布した場合，その煎汁は稲紋枯病菌菌糸の伸長を 2,4-D の濃度 5×10^{-2}%の時，最もよく促進する．

4．2,4-D 散布後，接種までの経過日数と稲紋枯病発生との関係を調べたが，散布後1，3，5，7日目ともに，その差が認められなかつた．

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Résumé

This paper deals with the results of the writers' experiments on the effects of 2,4-dichlorophenoxy acetic acid upon the mycelial growth and pathogenicity of *Hypochnus sasakii*.

1. The size and number of diseased spots of sheath blight of rice plant by *Hypochnus sasakii* more apparently increased in 2,4-D sprayed plots than in control plot.

2. The pathogenicity of the fungus in the diseased spots was stronger in 2,4-D sprayed plots than in control.

3. The growth of the mycelium of *Hypochnus sasakii* was stimulated in the decoction of rice plants which had been sprayed with 2,4-D at the concentration of 5×10^{-2} per cent.

4. Time after the spraying of 2,4-D to the rice plant has no definite influence to the size and number of diseased spots within the limit of the writers' experiments.

植物病害研究 (Shokubutsu Byogai Kenkyu, Kyoto) 6(4) : 136～138, 1959

微生物に対する放射線照射の影響（第6報）

胡瓜炭疽病菌分生胞子の生残曲線について*

正　子　　朔**

Hajime Masago : Studies on the effects of the radiation on microorganisms (6) On the survival curves of *Colletotrichum lagenarium* irradiated with ultraviolet light

1．緒　　言

第5報*** において筆者は，稲胡麻葉枯病菌分生胞子の生残曲線を解析して，分生胞子個体間における成長程度の変異が見かけの生残曲線あるいは殺菌所要エネルギーに大きな影響を与えていることを報じた．さらに筆者は，細菌の生残曲線に見られる直線的変化が胡麻葉枯病菌に見られない理由を考察したが，本報においては胞子個体間に比較的外形的変異が少なく，かつ成長程度による分散度も少ないと見做される炭疽病菌を対照として，稲胡麻葉枯病菌における考察の裏付けを行つた．

終始御懇篤な御指導を賜り，論文校閲の労をいただいた恩師赤井重恭教授に深甚なる謝意を表する．

2．実験結果

供試菌として研究室保存の胡瓜炭疽病菌（*Colletotrichum lagenarium* (Pass.) Ell. et Halst.）を用い，紫外線処理は常法[2]に従つたが，殺菌紫外線出力は 1020μw/cm^2 であつた．

第1表　単一胞子褥から得た分生胞子の発芽におよぼす紫外線の影響
The influence of ultraviolet irradiation on the germination of conidia from a sporodochium.

Time irradiated sec	First trial			Second trial		
	Conidia measured	Conidia germinated	Percent [a] germination	Conidia measured	Conidia germinated	Percent [a] germination
0	288	283	98.26%	295	290	98.30%
	318	313	98.42	300	294	98.00
	321	316	98.44	285	281	98.59
10	278	272	97.84	312	307	98.39
	308	303	98.37	298	293	98.32
	325	317	97.53	288	282	97.91
20	398	390	97.98	240	233	97.08
	242	236	97.52	283	270	95.40
	328	316	96.34	250	240	96.00
30	400	376	94.00	270	254	94.07
	309	291	94.17	290	273	94.13
	410	382	93.17	310	292	94.19
40	363	351	96.69	341	322	94.42
	286	270	94.40	325	307	94.46
	306	288	94.11	315	305	96.82
50	380	0	0	340	0	0
	350	0	0	350	0	0
	345	0	0	340	0	0
60	390	0	0	350	0	0
	360	0	0	360	0	0
	368	0	0	350	0	0

[a] measured after 22 hours at 28°C

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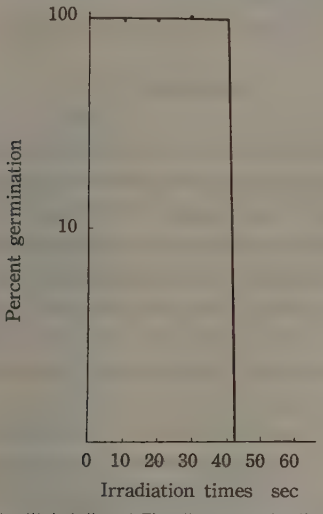
*** 未発表

1959年12月5日受領

A．単一胞子褥からの分生胞子試料についての照射実験

20日間28°Cにおいて玉蜀黍寒天上に培養して，形成した炭疽病菌の単一胞子褥から，1白金耳宛の胞子をとり，Czapek 氏液 2cc 中に懸濁せしめてスライド上に点滴後，紫外線処理を行つた．18時間28°Cに保つた後の発芽状況は第1表のごとくである．

上表ならびに第1図から明らかなように，50秒の照射によつて胞子は急激に殺され，発芽能力を喪失する．



第1図　炭疽病菌の胞子発芽におよぼす紫外線照射の影響
Influence of ultraviolet irradiation upon the conidia germination of *Colletotrichum lagenarium*

B．多数の胞子褥から得た分生胞子を混合した試料についての照射実験　20日間28°C下で培養後，5日間10°C附近に放置した試料の任意の胞子褥から胞子をとり，混合して前記Aと同様の処理を行つた．結果は第2表の通りである．

この結果から明かなように，多数の胞子褥から得た分生胞子では生残曲線がAの場合のように屹立せず，稲胡麻葉枯病菌分生胞子の場合のような曲線が得られる．

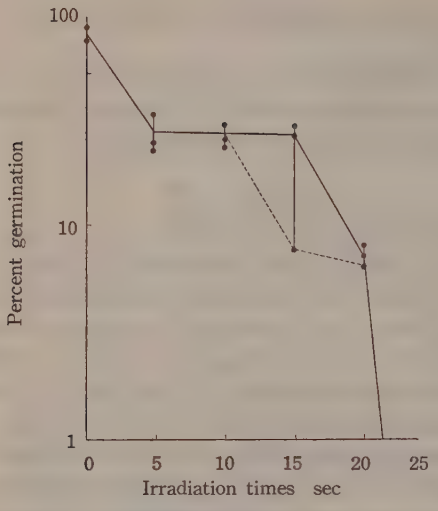
3．考　　察

胡瓜炭疽病菌の胞子には，その長径，短径ともに変異が少なく，検鏡によると透明な胞子膜を有する．この場合，形の上の変異が少ないから紫外線抵抗は稲胡麻葉枯病菌の場合と異つて，ほとんどすべての胞子が同じ抵抗力を有するものと考えてよかろう．第1表に示したように，供試胞子の致死点は40秒～50秒にあるものと考えられる．すなわち40秒として，40800μw-sec/cm² のエネルギーが各胞子に蓄積される迄は致死量に達せず，その量に達して急激に死滅する．この場合，照射量に対して死滅胞子数が変動しないのは，各胞子がある一定の弾着を得て後に，致死現象が始まることを意味する．40～50秒をさらに詳細に調査することによつて，この部分が直線的に（単的の場合）あるいは曲線的に（多的の場合）変化することがわかるであろうが，対照胞子が全く均一なものである以上，Lea の説く標的説[1]に従えば，著しく切り立つた直線になるのである．換言すれば，この水平部の長い程，供試菌胞子の抵抗力が大となり，直線部が切り立つほど，胞子が均一であることになる．第2表におけるごとく，混合胞子に照射した場合には，その生残曲線は若干の傾斜と段階性とを示している．しかし25秒で完全に殺されているのは，室温に放置したために，衰弱を来したものと見做される．炭疽病菌は，培養中に種々変異し，胞子の形成条件も複雑で不明の点が多い．第2図における生残曲線の段階性は Lea の説く標的の数に関係あるものと考えられるが，今後の研究によつて詳細を確めたいと思う．

第2表　2，3の胞子褥の分生胞子発芽におよぼす紫外線の影響
The influence of ultraviolet irradiation upon the germination of conidia from several sporodochia

Time irradiated sec	First trial			Second trial			Third trial		
	Conidia measured	Conidia germinated	Percent germination a)	Conidia measured	Conidia germinated	Percent germination a)	Conidia measured	Conidia germinated	percent germination a)
			%			%			%
0	191	141	73.27	200	150	75.00	220	183	83.18
5	219	49	22.37	250	61	24.40	513	170	33.13
10	280	67	23.92	224	55	24.45	177	52	29.38
15	214	60	28.03	289	75	25.95	289	21	7.26
20	162	13	8.02	202	14	6.93	264	16	6.06
25	200	0	0	200	0	0	250	0	0

a) measured after 22 hours at 28°C



第2図 炭疽病菌の2，3分生子褥から得た胞子の発芽におよぼす紫外線照射の影響
Influence of ultraviolet irradiation upon the germination of conidia obtained from several sporodochia of *Colletotrichum lagenarium*

4．摘 要

1．本報告においては，分生胞子の形態に比較的変異が少ないと見られる胡瓜炭疽病菌を対照とし，紫外線照射による影響を研究した．

2．炭疽病菌は胡麻葉枯病菌よりもはるかに紫外線抵抗が小さい．*

3．Dose-Response 曲線は Sigmoid 曲線にはならないで，低線量ではほとんど変化なく，ある線量で急激に生残率が低下する曲線になる（第1図）．

4．2，3の胞子褥から得た胞子を混合して照射すると前項の現象が重畳したような階段的な曲線が得られる（第2図）．

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Summary

In the present paper the writer described on the resistance of conidia of *Colletotrichum lagenarium* to ultraviolet light. Conidia obtained from a sporodochium of this fungus were comparatively uniform in size compared with that of *Cochliobolus miyabeanus*. Most of the conidia had been killed before the energy reached to 40,000μw-sec/cm^2, so that the survival curve dropped suddenly at this point. However, in the survival curve of conidia collected from several sporodochia a few discontinuous points were observed. This phenomenon seems to be due to the difference in resistance of conidia obtained from sporodochia at different growing stages. It should be confirmed whether the difference in resistance is based on targets in cells which Lea suggested.

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培地中のマレイン酸ヒドラジッドが植物病原菌の菌糸伸長，分生胞子形成および発芽におよぼす影響

青木嘉夫・田中国夫*

Yoshio Aoki and Kunio Tanaka:
Effect of maleic hydrazide in culture media on the mycelial growth, sporulation, and spore germination of various phytopathogenic fungi

植物生長抑制剤であるマレイン酸ヒドラジッド（略してMH）は，タバコ，トマトなどの腋芽の伸長，玉葱，馬鈴薯などの貯蔵中の発芽，腐敗などの防止に用いられているが，これが植物病原菌におよぼす影響はまだ明らかでない．最近西田，岡野[1]はタバコ炭疽病菌，腰折病菌，白絹病菌および疫病菌について培養基上でMHがこれらの菌の菌糸伸長を抑制し，あるいは栄養吸収に変化をおよぼし，またMH処理をしたタバコ苗が炭疽病および腰折病に対して抵抗性を増加することを報告している．本報告は数種の植物病原菌の発育におよぼすMHの影響を実験したものである．

植物病原菌の菌糸伸長におよぼす影響　供試MHは市販庵原農薬株式会社製の有効成分，マレイン酸ヒドラジッド30%（マレイン酸ヒドラジッド，ジエタノール塩として58%）のものであつて，供試菌は第1表記載の5種である．培地は蔗糖2%加用馬鈴薯汁寒天にMHを加えて30分間蒸気殺菌したものであつて，ペトリ皿に分注，固化後，予め7日間蔗糖2%加用馬鈴薯汁寒天上に平面培養した菌叢（直径約2mm）を接種した．しかし *C. centrifugum* のみは培養後1ヶ月の菌核を用いた．これらのペトリ皿は28°Cの定温器中に収め，2日後から毎日菌叢の直径を測定した．結果は第1表の通りである．

上表の如く供試菌はMH（0.15～0.9%）の添加によつて菌糸伸長を抑制される．その程度は菌の種類によつて異るが，白絹病菌が最も著しく，MH，0.9%の添加の場合には4日後に至つてもなお発芽せず，6日後に漸く半数が

第1表　供試病原菌の菌糸発育におよぼすMHの影響（3回実験平均結果）

供試菌	MH濃度 %	菌叢直径 mm 培養日数 2	3	4	5	1日当り菌糸伸長量 mm 培養期間(日) 2～3	3～4	4～5
Piricularia oryzae	0.9	10	16	22	27	6	6	5
	0.6	15	22	30	35	7	8	5
	0.3	17	27	38	47	10	11	9
	0.15	21	31	43	54	10	12	11
	0	21	38	51	66	17	13	15
Pyrenophora avenae	0.9	6	10	13	15	4	3	2
	0.6	9	15	22	27	6	7	5
	0.3	13	25	39	51	12	14	12
	0.15	14	31	43	55	17	12	12
	0	20	35	53	66	15	18	13
Corticium centrifugum	0.9	0	0	0	—	0	0	0
	0.6	5	16	34	—	11	18	—
	0.3	11	35	61	—	24	26	—
	0.15	18	51	79	—	33	28	—
	0	24	68	—	—	44	—	—
Phyllosticta lycopersici	0.9	14	22	30	39	8	8	9
	0.6	14	23	32	42	9	9	10
	0.3	17	26	37	44	9	11	7
	0.15	17	27	37	46	10	10	9
	0	19	29	39	48	10	10	9
Pleospora herbarum	0.9	13	21	24	35	8	3	11
	0.6	17	28	27	46	11	9	9
	0.3	22	33	44	55	11	11	11
	0.15	23	35	44	60	12	9	16
	0	27	39	52	66	12	13	14

第2表　分生胞子形成におよぼすMHの影響（3回実験平均結果）

供試菌	MH濃度 % 0.9	0.6	0.3	0.15	0
Piricularia oryzae	** 0	0	0	0	1
Pyrenophora avenae	0	0	0	0-1	2
Phyllosticta lycopersici	2-3	2-3	3	4	5
Pleospora herbarum	0	0	0	0	2-3

** 胞子形成程度（培養10日後）

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1）西田耕，岡野秋盛：日本植物病理学会報　22(1)：16，1957

1959年12月7日受領

発芽した．しかして *Piricularia oryzae* は約50%，*Pyrenophora avenae* は約30%の発育抑制を受ける．

次にそれら菌叢上の分生胞子形成程度を調べたが，胞子密度は10日間培養の菌叢を任意の個所から数ケ所適宜に白金環で抜き取つて顕微鏡下で測定し，0～5の5段階としてあらわした（第2表）．

上記の如く，分生胞子形成は *Phyllosticta lycopersici* を除いて他は殆んど認められない．

分生胞子発芽におよぼす影響　*Piricularia oryzae*, *Pyrenophora avenae*, *Pleospora herbarum* の3菌を用いて分生胞子の発芽試験を行つた．予め約1ケ月間庶糖2%加用馬鈴薯汁寒天上に培養した上記3菌の胞子懸濁液（蒸留水）を蒸留水で稀釈し，1視野中（倍率10×10）に胞子が約10個認められるようにした．その1ccに所定濃度のMHを加えて試験管中に注ぎ，定温器中（28°C）に24時間試験管を傾斜して保つた．結果は第3表の通りである．

第3表　分生胞子の発芽におよぼすMHの影響（3回実験平均結果）

MH濃度 %	*Piricularia oryzae* %		*Pyrenophora avenae* %		*Pleospora herbarum* %	
0.9	55.4	*（65）	22.6	*（32）	20.8	*（32）
0.6	64.5	（76）	34.9	（50）	28.6	（42）
0.3	72.7	（86）	50.4	（72）	34.7	（53）
0.15	77.7	（91）	56.3	（80）	54.2	（83）
0	85.0	（100）	70.4	（100）	65.1	（100）

*　括弧内の数字は標準区に対する百分率

上記の如く分生胞子発芽に関しても，添加量の増加に従つて発芽率が減少する．特に高濃度添加区においては発芽管の伸長が著しく悪い．

以上の如く，MHは実験の濃度範囲内において，供試病原菌の発育を阻害する．